Biochimica et Biophysica Acta, 455 (1976) 1–7

BBA 77488

LIPID-MEDIATED PROTEIN INTERACTION IN MEMBRANES*

S. MARČELJA

Institut za fiziku Sveučilišta, Zagreb (Yugoslavia) and Department of Applied Mathematics, Institute of Advanced Studies, Research School of Physical Sciences, Australian National University, Canberra 2600 (Australia)

(Received April 6th, 1976)

SUMMARY

This study describes the effects ensuing from a non-specific interaction between membrane integral proteins and the surrounding lipids. The results are obtained using an appropriate molecular field theory to describe the ordering of membrane lipids.

The modification of the lipid structure near a protein molecule, while most pronounced within the annulus of the first neighbour molecules, extends two or three layers beyond the annulus. The ordering of lipids within the annulus has a modified temperature dependence, and becomes a continuous function of temperature for low lipid/protein ratios.

The change in order of lipid molecules surrounding a protein leads to an indirect, lipid-mediated interaction between membrane integral proteins. This interaction depends sensitively on the bulk lipid order. Under favourable circumstances, it gives rise to protein aggregation.

INTRODUCTION

A number of recent experiments have demonstrated a structural change in the phospholipid environment of membrane integral proteins. The initial evidence was obtained from the shape of the ESR spectrum of a spin label dissolved in model membranes containing a known fraction of a single protein [1, 2]. An alternative spin label experiment [3] used the reduction kinetics of the label to obtain information on the enzyme environment. More recent direct evidence [4, 5] comes from the assays of the ATPase activity in model membranes with varying composition and external conditions.

The evidence obtained using either of the mentioned techniques shows that the most important change in lipid structure is restricted to the "annulus" of the first neighbour phospholipid molecules surrounding a protein. In an attempt to draw a picture of the phenomenon, different authors [1, 3, 6] show a protein molecule surrounded by a rigid annulus of the first neighbour phospholipids. The subsequent lipid layers are shown as not perturbed by the presence of a protein.

* Presented in part at the 5th International Biophysics Congress, Copenhagen 4–9 Aug. 1975.

The perturbation of lipid ordering near a protein molecule may be calculated by extending a molecular field method introduced in our earlier model of chain ordering in bilayer membranes [7]. We have selected that model because it currently gives the most realistic picture of the phospholipid chain order [8]. The generalization required to include the inhomogeneous spatial distribution of lipid order is easily accomplished. We proceed to calculate the detailed structure of the annulus, and introduce an entirely novel concept of the indirect, lipid-mediated protein interaction.

STRUCTURE OF THE ANNULUS

We start with a brief introduction of the modified molecular field model. All the necessary details which are omitted here are described in refs. 7 and 8.

In the ordered phase of lipid bilayers membranes, rigid hydrocarbon chains are arranged in a two-dimensional hexagonal lattice [9]. The spatial order is partially retained in the liquid phase, and we shall use a lattice model to describe the positions of hydrocarbon chains near a protein molecule. The chains will be assumed to occupy the sites of a planar hexagonal lattice. At each lattice site j, one can define the order parameter η_j

$$\eta_j = \left\langle \frac{1}{n} \sum_m \left(\tfrac{3}{2} \cos^2 \nu_m - \tfrac{1}{2}\right) \right\rangle_j \tag{1}$$

ν_m describes the orientation of a chain segment and the sum runs over the chain which is assumed to have n segments taking different orientations. The statistical mechanics averaging is performed separately for each site j.

In the molecular field approximation, the molecular field at each site i can be obtained by taking an average over the order of the first neighbours

$$\Phi_i = \tfrac{1}{6} \sum_{j=1}^{6} V_0 \phi_j, \tag{2}$$

where

$$\phi_j = \langle (n_{\mathrm{tr}}/n^2) \sum_m \left(\tfrac{3}{2} \cos^2 \nu_m - \tfrac{1}{2}\right) \rangle_j \tag{3}$$

and n_{tr}/n is the fraction of trans states. Φ_i describes the strength of the molecular field acting to orient the molecule on site i. The coupling constant V_0 used in refs. 7 and 8 is 680 cal/mol and 590 cal/mol, respectively. In the subsequent numerical analysis we have retained the former value for V_0.

Within the model, a single protein molecule is described by a geometrical shape taking up a certain number of lipid chain sites. In the present work we have considered simple hexagonal-shaped proteins, with a side length of 3, 4 or 5 lipid sites. The picture of the model is shown in Fig. 1.

When a lipid chain on the site i is neighbouring a protein on the site j, a term $V_0 \Phi_j$ in the sum of Eqn. 2 is replaced by a constant V_{lp} describing the lipid-protein interaction. In the subsequent calculation, V_{lp} is the single free parameter. The energy of a chain at the site i depends on the chain conformation, the value of the molecular field Φ_i and the lateral pressure P within the bilayer

$$E_i(\Phi_i, P) = E_{\mathrm{int}} - \Phi_i (n_{\mathrm{tr}}/n^2) \sum_m \left(\tfrac{3}{2} \cos^2 \nu_m - \tfrac{1}{2}\right) + PA. \tag{4}$$

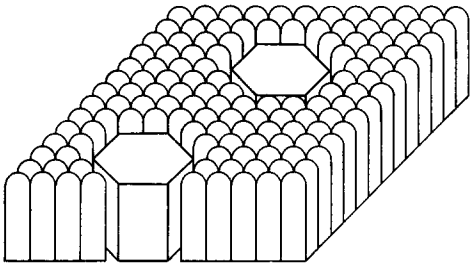

Fig. 1. A part of the model system consisting of two protein molecules surrounded by lipids.

For a given chain conformation, E_{int} is the internal energy, A the cross sectional area and n_{tr}/n the fraction of trans states. The partition function for a chain at the site i is

$$Z_i = \sum_{\text{all conformations}} \exp\left[-E_i(\Phi_i, P)/k_B T\right], \tag{5}$$

and the average values of Φ_i defined in Eqn. 3 are obtained from the self-consistent system of equations

$$\phi_i = (1/Z_i) \sum_{\text{all conformations}} (n_{\text{tr}}/n^2) \sum_m (\tfrac{3}{2}\cos^2 \nu_m - \tfrac{1}{2}) \exp\left[-E_i(\Phi_i, P)/k_B T\right] \tag{6}$$

Since Φ_i depends on the values of ϕ_j at the neighbouring sites, if M is the number of lipid molecules, Eqn. 6 is a system of M coupled non-linear equations. The system can be solved numerically by iteration. The expressions for thermodynamic functions are obtained by summation of the contributions from all lipid molecules. In the calculation of the internal energy, care must be taken to count properly the contribution from lipid-protein bonds

$$U = \sum_i \left[E_{\text{int}} - n\Phi_i \phi_i/2\right] + V_{lp} \sum_{\substack{\text{l-p} \\ \text{bonds}}} n\phi_i/12. \tag{7}$$

The first term counts the contribution from lipid-protein bonds with a factor of $\frac{1}{2}$, the second term is therefore added to obtain the correct value.

The entropy and the Gibbs free energy are given by

$$\begin{aligned} -TS &= -k_B T \ln Z - k_B T^2 \partial \ln Z/\partial T \\ &= \sum_i \left[-k_B T \ln Z_i + n\Phi_i\phi_i - E_{\text{int},i} - PA_i\right] \end{aligned} \tag{8}$$

$$G = U - TS + PA, \tag{9}$$

where $E_{\text{int},i}$ and A_i are, respectively, the average internal energy and the average cross-sectional area of the lipid molecule, and A is the total lipid area*. Protein molecules are assumed to remain in a single conformation, and consequently there is no protein contribution to the total entropy.

The results obtained with a 25×35 lattice model are presented in Figs. 2–4. In all numerical calculations, the chains were assumed to have 12 carbon atoms corre-

* In ref. 7, the PA term was omitted from both Eqns. 8 and 9. None of the results were affected. We are very grateful to Professor A. Caille and Professor A. Rapini for pointing out the omission.

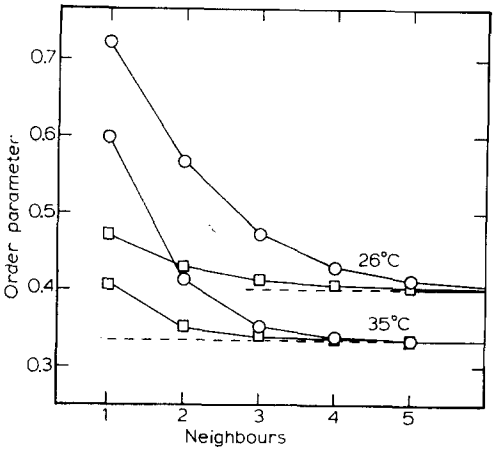

Fig. 2. Profiles of the annulus: order parameter of phospholipid chains as a function of the distance form the protein. Although the change is largest for the first neighbours, further layers are also affected. ○, $V_{1p} = 0.9\ V_0$; □, $V_{1p} = 0.5\ V_0$; – – – –, bulk value of the order parameter.

sponding to Fig. 4 of ref. 7. The bulk phase transition temperature for that membrane is 25.2 °C. The protein molecule is represented by a hexagon with a varying side length.

Let us first consider an isolated protein. The average values of the order parameter η for the first neighbours (the annulus), second neighbours, etc. are shown in Fig. 2. All the results for order parameter show very minor dependence on protein size and the side length of three lipid sites was chosen for the calculation of Fig. 2.

In these, as well as in all other calculated order parameter profiles, the disturbance caused by a protein extends to the second or the third neighbours. Compared to the change in order of the first neighbours the effect is small, and not likely to be detected by the synthesis of the ESR spectra [1, 2]. The slower decay of the perturbation at a temperature closer to the phase transition reflects a typical increase in order parameter correlation length.

The calculations have also been performed for temperatures below the lipid phase transition, where similar results are obtained. However, as explained in ref. 7, the model does not treat accurately the long-range correlations important for stiff chains and the results below the lipid phase transition are less reliable.

As the temperature is varied, the order of the lipid molecules comprising the annulus changes, and it is most likely that it is this change that controls the ATPase activity in the experiments of refs. 4 and 5. A typical experiment is performed with a membrane containing a high concentration of protein. To describe such a situation, we have also studied the behaviour of a system consisting of a periodic protein lattice embedded in the lipid matrix. Each protein molecule was assumed to retain only its own boundary lipid. The mathematical solution of the self-consistency Eqn. 6 is obtained using the periodic boundary conditions. The results obtained with a protein side length equal to five (covering 37 lipid sites) are shown in Fig. 3 for both an isolated protein and a membrane containing high protein concentration. It is seen that in the latter case the change of order with temperature is continuous. For weaker

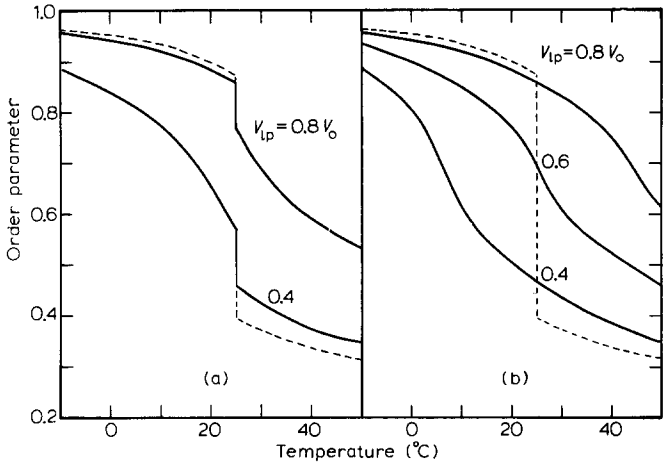

Fig. 3. Order parameter of the first neighbour annulus as a function of temperature. (a) Isolated protein. (b) Protein lattice; – – –, order parameter for bulk lipids.

lipid-protein interaction ($V_{lp} = 0.4\ V_0$), the region of rapid change in lipid order is shifted to temperatures below the bulk lipid phase transition. Such a behaviour was indeed observed in the studies of the activity of ATPase incorporated in single component bilayers [4].

PROTEIN-PROTEIN INTERACTION

When two protein molecules within the membrane approach each other, the regions with modified lipid structure surrounding each molecule start to overlap. This overlap results in order parameter configurations reminiscent of the potential field around two charges of the same sign. It leads to an indirect, lipid-mediated interaction between the proteins.

To find the strength and the temperature dependence of the indirect protein interaction, we calculate the free energy G of the combined lipid-protein system as a function of the protein separation x. According to the laws of statistical mechanics, the free energy represents the effective protein-protein potential, and the derivative $\partial G/\partial x$ is the force acting between the molecules. Numerical results for different temperatures and different values of V_{lp} are shown in Fig. 4. The proteins were assumed to have a side length of three lattice sites. For larger proteins, the interaction strength was found to be approximately proportional to the side length.

Each protein molecule perturbs the structure of the surrounding lipids. However, if two protein molecules are close or in contact, the total perturbation of the lipid structure decreases. This causes a net attractive force between the proteins. At a distance where protein molecules come into contact a contribution from the direct protein-protein interaction has to be added to the free energy balance. Depending on the relative strength of the direct protein-protein interaction in comparison with the lipid-protein interaction, proteins may remain separated by a layer (or two) of lipids or come into contact. The contribution from the rather specific direct protein-protein

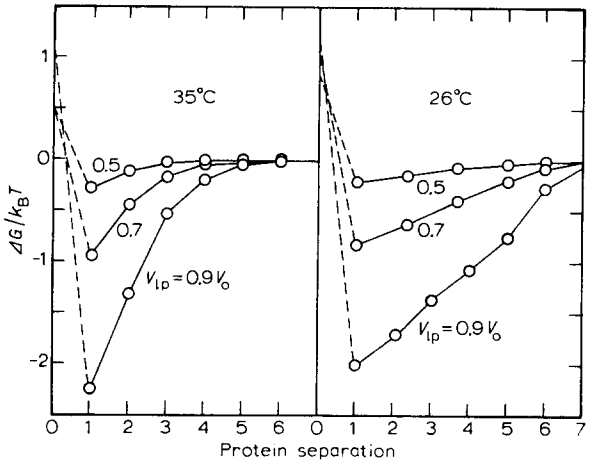

Fig. 4. Change in the free energy ΔG calculated as a function of protein separation. The separation is shown as a number of lipid layers between protein molecules. For proteins in direct contact, dashed lines show the value of $\Delta G/k_BT$ without the contribution from direct protein-protein interaction (see text).

interaction, which is not shown in Fig. 4, may become a dominant force for proteins in contact.

It should be noted that the present work considers only the hydrophobic interaction of proteins with lipid chains, whilst the ionic interaction of polar headgroups with the protein has not been taken into account. At present, no experimental information on the latter interaction is available. However, such an ionic interaction needs to be considered together with the present results for the hydrophobic interaction as an important component in determining the total protein interaction as well as the protein function. An even more complicated situation arises for protein molecules protruding out of the bilayer structure. The charge properties of such proteins and the induced local modifications in water structure will clearly be of great importance in determining the total protein interaction. Finally, the van der Waals interaction and the elastic interaction of conically shaped protein molecules discussed by Gruler [10] should also be considered under appropriate circumstances.

As the temperature of the membrane decreases towards the phase transition of the lipids, the lipid order parameter correlation length and consequently the range of the protein interaction increase (Fig. 4). The increase of interaction range leads in turn to the increased protein-protein correlation, and eventually to protein aggregation. Using the above model one can study the protein aggregation in membranes as a condensation of a two-dimensional protein gas in the plane of the membrane. The role of an effective potential between the proteins $V(r)$ is taken here by the free energy difference $\Delta G(r)$. Whether the aggregation will be favourable or not will depend on the function $V(r)$ and the protein density. (From Fig. 4, typical numerical values for $V(r)$ are comparable to the thermal energy k_BT.) For example, the condensation can be described with the van der Waals equation with the approximate values of the van der Waals parameters a and b obtained from the second virial coefficient (see for example, ref. 11).

The value of the protein-protein interaction potential in biological membranes can be obtained from the currently available electron micrographs showing protein particles within the membrane (Naghizadeh, J., unpublished). The experimental information about the protein interaction is contained in the radial distribution function of proteins, obtained from the analysis of the electron micrographs. Preliminary results of such a study (Naghizadeh, J., unpublished) support the picture of lipid-mediated protein interaction in biological membranes.

ACKNOWLEDGEMENTS

I thank Professor E. Sackmann and Professor J. Naghizadeh for stimulating discussions. This work was supported in part by the Scientific Research Council of the Socialist Republic of Croatia.

REFERENCES

1 Jost, P. C., Griffiths, O. H., Capaldi, R. A. and Vanderkooi, G. (1973) Proc. Natl. Acad. Sci. U.S. 70, 480–484
2 Jost, P. C., Griffiths, O. H., Capaldi, R. A. and Vanderkooi, G. (1973) Biochim. Biophys. Acta 311, 141–152
3 Stier, A. and Sackmann, E. (1973) Biochim. Biophys. Acta 311, 400–408
4 Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1974) Biochemistry 13, 5501–5507
5 Warren, G. B., Houslay, M. D., Metcalfe, J. C. and Birdsall, N. J. M. (1975) Nature 255, 684–687
6 Vignais, P. M. and Deveaux, P. F. (1975) in Membrane-bound Enzymes (Martonosi, A., ed.), Plenum, New York
7 Marčelja, S. (1974) Biochim. Biophys. Acta 367, 165–176
8 Schindler, H. and Seelig, J. (1975) Biochemistry 14, 2283–2287
9 Tardieu, A., Luzzati, V. and Reman, F. C. (1973) J. Mol. Biol. 75, 711–733
10 Gruler, H. (1975) Z. Naturforsch. 30c, 608–614
11 Isihara, A. (1971) Statistical Physics, p. 113, Academic Press, New York